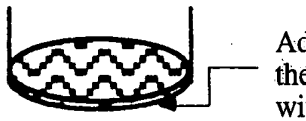
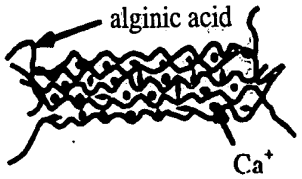
FIG. 1

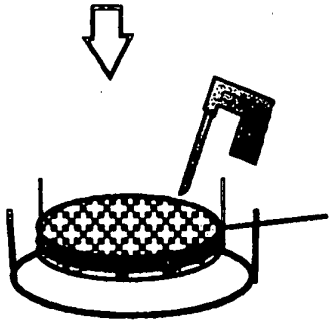


Add 1ml of 1% sodium alginate solution to the cell of which bottom is a porous membrane with a pore size of 3.0μm.

Allow 0.1M $CaCl_2$ to soak from underneath and leave 1 hour at room temperature, thereby to occur gelation.



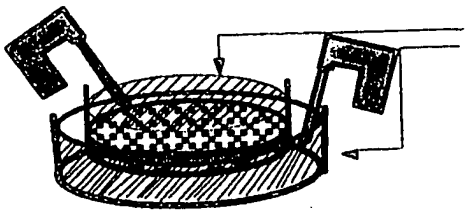
Fine structure of calcium alginate gel



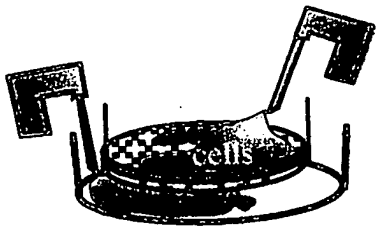
Add 0.5ml of a collagen solution diluted to 3% with D-MEM medium to prepare a thin layer.

Gelation in CO_2 incubator at 37℃ for 20 minutes.

FIG. 2

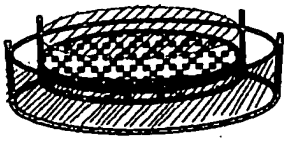


Add D-MEM medium, 2 ml into the cell and 3 ml to a schale, and then infiltrate overnight.



Remove the medium in the cell or schale,
spread 10,000 cells (0.5ml) of fibroblast
collected by trypsin treatment,
and add 3ml of D-MEM medium to the schale.

Leave in a 0.5% CO_2 incubator for about 1 hour at 37℃ to allow the attachment of fibroblast.



Exchange the medium on Day 2 after culture,
and further culture for a period of one day
to form a confluent cell monolayer.

Laminate structure of gel and cultured cell monolayer

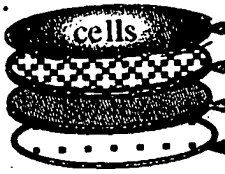
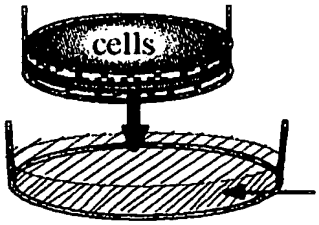
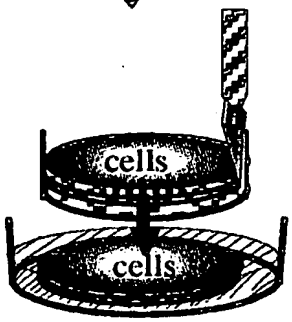


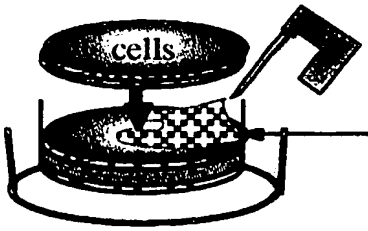
FIG. 3



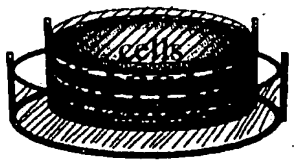
Soak the cell into 0.1M EDTA solution to dissolve the calcium alginate gel and liberate it from the membrane filter



Remove extra water from the cell by suction, insert a scalpel through the inner wall of the cell to hollow the filter, thereby suspending a cell sheet of collagen gel in D-MEM medium.



Add 0.5ml of a collagen solution onto the gel sheet which is not suspended, and overlay a gel formed in the same manner as above



Culture three cell layers laminated with a sandwiched collagen gel, in D-MEM medium.